20 CARISOPRODOL AND MEPROBAMATE QUANTITATION BY GC/FID

20.1 Summary

20.1.1 Biological samples are buffered with phosphate buffer (pH 7) and extracted with a mixture of hexane and ethyl acetate. The extract is washed with hexane and reconstituted with toluene/hexane/isoamyl alcohol. An aliquot is injected into a GC equipped with an FID detector for quantitation of carisoprodol and meprobamate. The aliquot can be subsequently injected into a GCMS for confirmation, if necessary.

20.2 Specimen Requirements

20.2.1 200 µl biological fluid or comparable amount of tissue dilutions/homogenates
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20.3 Reagents and Standards

20.3.1 Carisoprodol

20.3.2 Meprobamate

20.3.3 Cyclopal (cyclopentalbarbital)

20.3.4 Trimethyl sulfonium iodide

20.3.5 Disodium phosphate (Na_2HPO_4)

20.3.6 Monosodium phosphate (NaH_2PO_4)

20.3.7 Silver oxide

20.3.8 Hexane

20.3.9 Isoamyl alcohol

20.3.10 Methanol

20.3.11 Toluene

20.3.12 Ethyl acetate

20.3.13 Acetonitrile

20.3.14 N-chlorobutane

20.4 Solutions, Internal Standard, Calibrators and Controls

20.4.1 0.1 M disodium phosphate: Weigh 1.70 g of disodium phosphate and transfer to 1L volumetric flask. QS to volume with dH_2O.

20.4.2 0.1 M monosodium phosphate: Weigh 12.14 g monosodium phosphate and transfer to a 1 L volumetric flask. QS to volume with dH_2O.

20.4.3 0.1 M sodium phosphate buffer (pH 7.0): Mix 500 mL 0.1 M disodium phosphate with approximately 250 mL 0.1 M monosodium phosphate. Adjust pH to 7.0 ± 0.1 with 0.1 M monosodium phosphate (lowers pH) or 0.1 M disodium phosphate (raises pH).

20.4.4 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol

20.4.5 Hexane/ethyl acetate (50:50, v:v) extraction solvent: Mix 50 mL hexane with 50 mL ethyl acetate

20.4.6 Trimethyl sulfonium hydroxide derivatizing reagent. Add 6.12 g trimethylsulfonium iodide, 7.39 g silver oxide, and 15 mL methanol to a 25 mL teflon capped test tube covered with aluminum foil (light sensitive reaction). Rotate for four or more hours, centrifuge, and decant the supernatant to an aluminum foil covered test tube. Store in freezer.

20.4.7 Methanol/ dH_2O (50:50, v:v) Mix 50 mL methanol with 50 mL dH_2O

20.4.8 Drug stock solutions:

20.4.8.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and QS to volume with methanol.

20.4.8.2 Internal standard solution (methylated cylopal (cyclopentalbarbital)): To 2 mL of 1 mg/mL cyclopal (in methanol), add 1 mL TMSH. Cap and heat at 60° C for 2 hours. Let sit at room temperature overnight. Evaporate under nitrogen. Reconstitute with 10 mL methanol/ dH_2O (50:50, v:v).

20.4.9 Blood calibrators, standards, and controls preparation:

20.4.9.1 To prepare the calibration curve, pipet the following volumes of 1 mg/mL carisoprodol and meprobamate stock solutions into appropriately labeled 13 x 100 mm screw cap test tubes

- 100 mg/L Calibrator — 300 µL each of carisoprodol and meprobamate
- 50 mg/L Calibrator — 150 µL each of carisoprodol and meprobamate
- 20 mg/L Calibrator — 60 µL each of carisoprodol and meprobamate
- 10 mg/L Calibrator — 30 µL each of carisoprodol and meprobamate
- 5 mg/L Calibrator — 15 µL each of carisoprodol and meprobamate
- 2 mg/L Calibrator — 6 µL each of carisoprodol and meprobamate

20.4.9.2 Evaporate standards to dryness under nitrogen. Add 3 mL blank blood to each tube. Calibrators may be stored in refrigerator for up to 1 year after preparation.

20.4.9.3 Controls

20.4.9.3.1 Negative control. Blood bank blood (or comparable) determined not to contain carisoprodol or meprobamate

20.4.9.3.2 Positive control. In house control containing each analyte of interest from a different lot number or manufacturer than standards, or prepared by a chemist different than the one performing the extraction.

20.5 Apparatus

20.5.1 Agilent GC/MSD, Chemstation software (for confirmation, if necessary)

20.5.2 Agilent GC equipped with Flame Ionization Detector, Chemstation software, compatible computer & printer

20.5.3 Test tubes, 13 x 100 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps

20.5.4 Test tubes, 16 x 114 mm (10 mL) glass centrifuge, conicals

20.5.5 Centrifuge capable of 2,000 – 3,000 rpm

20.5.6 Vortex mixer

20.5.7 Evaporator/concentrator

20.5.8 GC autosampler vials and inserts

20.5.9 Test tube rotator

20.5.10 GC/FID parameters. Conditions may be changed to permit improved performance.

20.5.10.1 Oven program.

- Equilibration time: 0.50 minutes
- Initial temp: 110° C
- Initial time: 1.0 minutes
- Ramp: 20° C/min
- Final Temp: 260° C
- Final Time: 1.5 minutes
- Run Time: 15 minutes

20.5.10.2 Inlet.

- Mode: Splitless
- Temperature: 250° C
- Constant pressure: 25 psi
- Purge flow: 1.9 mL/min
- Total flow: 6.1 mL/min
- Injection volume: 1.0 µL

20.5.10.3 Detector.

- Temperature: 290° C
- Hydrogen flow: 50 mL/min
- Air flow: 450 mL/min
- Mode: Constant makeup flow
- Makeup flow: 45 mL/min

20.5.10.4 Column: HP-5 30 m x 0.25 mm x 0.25 µm.

20.5.11 GC/MSD parameters. Conditions may be changed to permit improved performance.

20.5.11.1 Acquisition Mode: Scan (50 – 550 amu)

20.5.11.2 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

20.5.11.3 Detector Temperature: 280° C

20.5.11.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110° C
- Initial time: 1 minutes
- Ramp: 10° C/min
- Final Temp: 290° C
- Final Time: 9 minutes
- Run Time: 28 minutes

20.5.11.4.1 Inlet

- Mode: Splitless
- Temperature: 270° C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

20.6 Procedure

20.6.1 Label clean 13 x 100 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.

20.6.2 Prepare calibrators and controls

20.6.3 Pipet 200µL of each calibrator, control, negative and case samples into appropriately labeled tubes.

20.6.4 Add 30 µL methylated cyclopal internal standard to each tube.

20.6.5 Add 0.5 mL sodium phosphate buffer (pH 7) to each tube.

20.6.6 Add 3 mL extract solvent (hexane/ethyl acetate) to each tube.

20.6.7 Cap and rotate tubes for 15 minutes.

20.6.8 Centrifuge at approximately 2500 rpm for 15 minutes. Transfer organic upper layer to clean 10 mL conical bottom centrifuge tubes. Discard lower layers.

20.6.9 Evaporate samples to dryness under nitrogen at 50-60° C.

20.6.10 Reconstitute samples with 0.2 mL acetonitrile. Vortex briefly.

20.6.11 Add 1 mL hexane to each tube. Vortex each sample for 30 seconds.

20.6.12 Centrifuge at approximately 2500 rpm for 5 minutes.

20.6.13 Aspirate (and discard) upper (hexane) layer.

20.6.14 Evaporate lower (acetonitrile) layer under nitrogen at 50-60° C.

20.6.15 Reconstitute samples with 75 µL of toluene/hexane/isamyl alcohol solvent or n-chlorobutane and vortex briefly

20.6.16 Transfer samples to appropriately labeled GC vials and inject 1-2 µl on GC-FID.

20.6.17 Save remainder of reconstituted samples for confirmation by GC-MSD (if not already confirmed).

20.7 Calculation

20.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios versus calibrator concentration.

20.8 Quality Control and Reporting

20.8.1 See Toxicology Quality Guidelines

20.9 References

20.9.1 in house development, T England